TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/US00/09685
International Filing Date:	April 11, 2000
Priority Date Claimed:	April 12, 1999
Title of Invention:	METHOD OF PRODUCING PROBE ARRAYS FOR BIOLOGICAL MATERIALS USING FINE PARTICLES
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) () is transmitted herewith (required only if not transmitted by the International Bureau).
   b) () has been transmitted by the International Bureau.
   c) (X) a copy of Form PCT/1B/308 is enclosed.
   d) () is not required, as the application was filed in the United States Receiving Office (RO/US).

5. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))

   a) () are transmitted herewith (required only if not transmitted by the International Bureau).
   b) () have been transmitted by the International Bureau.
   c) () have not been made; however, the time limit for making such amendments has NOT expired.
   d) (X) have not been made and will not be made.

6. (X) An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

7. (X) International Application as published.

8. (X) International Search Report.

9. (X) PCT Form PCT/IPEA/402..

10. (X) Drawing in eleven (11) pages.

11. (X) A return prepaid postcard.

12. (X) The following fees are submitted:

				FEES
	BASIC FEE			$690
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	28 - 20 =	8 ×	$18	$144
Independent Claims	7 - 3 =	4 ×	$80	$320
	TOTAL OF ABOVE CALCULATIONS		$	
	TOTAL FEES ENCLOSED			$1,154

13. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.

14. (X) A check in the amount of $1,154.00 to cover the above fees is enclosed.

15. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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Method of Producing Probe Arrays for Biological Materials Using Fine Particles

Background of the Invention

Field of the Invention

The present invention relates to probe arrays for use in detecting peptides, proteins and DNAs, diagnosing, and analyzing biological materials including DNAs; and methods and apparatuses to produce the same.

Description of the Related Art

For DNA analyses or DNA tests or diagnoses, amplification of a small amount of DNA, isolation and identification of the amplified DNA fragments, and other procedures are necessary. For DNA amplification, PCR (polymerase chain reaction) is widely used, in which an extremely small number of DNAs can be multiplied by several orders of magnitude so as to be detectable. On the other hand, for the isolation and detection of different DNAs, among other methods, a DNA sequencer and fragment analyzer, in which gel electrophoresis and fluorescence detection are combined, are used. However, electrophoresis becomes very labor-intensive as the number of samples or test items increases. Thus, a simple method using DNA probes is drawing attention, in particular, a DNA chip, in which many kinds of probes are immobilized on the surface of a solid to make a probe array which undergoes hybridization with the sample, then only specific DNAs are trapped on the surface of the solid and detected (Nature Medicine 2, 753, 1996).

The probe detection method is used also for the analysis of proteins or peptides or various biological materials which interact with them, and a peptide chip corresponding to the DNA chip is now being used. This kind of isolation and detection method, in which a peptide or DNA is immobilized on the surface of a solid and hybridization proceeds between the peptide or DNA and a sample, has long been known as a blotting method in which the presence of the target DNA or the like is detected by a probe immobilized on a membrane using radioactive labeling. However, the DNA chip, on which a large number of probes can be immobilized on a small area (1 cm^2) of the

surface of a solid such as glass or silicone, has the advantage in that only a small amount of sample is required, and a vast variety of probes can be used simultaneously. Methods for the production of DNA chips are divided broadly into two groups. In the first group, a DNA probe is synthesized one base at a time by a photochemical reaction on small segments (0.05 mm^2 to 0.2 mm^2) of a solid using the same photomasking technique as used for semiconductors or the like (Science 251, 767, 1991). In the second group, a synthesized DNA, PCR-amplified DNA, or DNA obtained by cloning is immobilized on a small segment of the surface of a solid for each segment of individual probes (Nature Biotech 16, 27, 1998). The latter has the advantage that a peptide chip or DNA chip with the required probes can be made relatively easily, and is the method of choice of many startup companies.

Summary of the Invention

A probe chip for biological materials, including DNA, is a highly anticipated to be used as a testing tool. However, for practical purposes, the following conditions have to be satisfied: (A) a small amount of a large variety of chips can be made at low cost, (B) a probe can be immobilized homogeneously, (C) data is highly reproducible and the chip is reusable, and (D) the chip can be heated to remove nonspecifically absorbed substances. However, problems remain: For example, (a) the probes are not consistent from one segment to another, (b) production is very labor-intensive, (c) very fine segmentation for immobilization is not possible, and (d) probes are not uniform; because (i) they are immobilized as liquid drops on the surface of a solid, and (ii) probes are positioned and immobilized simultaneously. Furthermore, (d) bind weakly with the surface of the solid and may dislodged upon heating, because (iii) many probe chips are immobilized by adsorption or the like.

In order to solve the aforementioned problems, immobilization of probes on the solid surface and alignment of the probes may be separated into two or more different steps to enable uniform DNA probes to be produced on the solid surface. The probes can be immobilized via covalent bonds, which are heat stable, therefore, nonspecifically absorbed substances can be appropriately removed by heating. Fine particles, used as the solid on which probes are immobilized, are aligned to produce a probe array having

segments of a suitable size. Any desired probe array can be readily produced by exchanging the aligned fine particles with the probes. Tweezers can be used to align fine particles having a diameter of about 0.3 mm but this method would be difficult for particles having a diameter of less than 0.1 mm. Therefore, in an embodiment, the present invention provides a method and an apparatus to produce a probe array, in which fine particles each held in a fine hole on a sheet are transferred and aligned in a capillary, a groove on a plate or the like. In an alternative method, fine particles are controlled to flow as individual particles into a liquid for transfer into a capillary to produce a probe array. Furthermore, in order to improve reproducibility in measurement, a multiple number of fine particles with a multiple number of probes are aligned for each probe to check any variation in test results to obtain highly reliable data.

For purposes of summarizing the invention and the advantages achieved over the prior art, certain objects and advantages of the invention have been described above. Of course, it is to be understood that not necessarily all such objects or advantages may be achieved in accordance with any particular embodiment of the invention. Thus, for example, those skilled in the art will recognize that the invention may be embodied or carried out in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objects or advantages as may be taught or suggested herein.

Further aspects, features and advantages of this invention will become apparent from the detailed description of the preferred embodiments which follow.

Brief Description of the Drawings

These and other features of this invention will now be described with reference to the drawings of preferred embodiments which are intended to illustrate and not to limit the invention.

Figure 1 is a conceptual view of a probe array chip comprising beads with probes aligned in a capillary.

Figure 2 is a conceptual view of a detection system to measure a bead array with probes retained in a capillary or the like.

Figures 3a-3g are fragmentary sectional views of an apparatus for bead alignment. Figure 3a is a conceptual view of bead feeding in an off-line state. Figure 3b is a conceptual view in which a bead is trapped in a hole. Figure 3c is a conceptual view in which a bead is moving into a capillary or the like. Figures 3d-3g show the subsequent steps.

Figures 4a and 4b are conceptual views of an apparatus for the groove-type bead alignment. Figure 4a is a perspective illustration. Figure 4b is a sectional view.

Figures 5a, 5b, and 5c are conceptual views of a method for producing a bead array using grooves and a movable valve. Figure 5c is a cross-sectional partial view.

Figure 6 is a conceptual view of a disk-type system for probe bead transfer.

Figure 7 is a conceptual view of a liquid flow-type bead array production method.

Figure 8 is a conceptual view of a bead array in which a large number of beads are separated with marker beads.

Figures 9a and 9b are conceptual views of a method of aligning probe beads using a sheet with holes.

Figures 10a, 10b, and 10c are conceptual views of a microtiter plate-type bead array holder. Figure 10a is a general view. Figure 10b is a sectional view. Figure 10c is a conceptual view for measurement.

Detailed Description of the Preferred Embodiment

The present invention includes a plurality of aspects and embodiments. In one aspect, a method for producing a probe array comprises the steps of: (a) selecting plural types of probes of interest; (b) immobilizing the plural types of probes on surfaces of different solid pieces, respectively; and (c) aligning the probe-immobilized solid pieces in a designated order to obtain a probe array for analyzing a sample solution passing therethrough. In the above, the probes may be polynucleotides, peptides, or proteins. In an embodiment, the solid pieces are beads which may be fine particles. Further, the alignment of the solid pieces may be a one-dimensional arrangement or a two-dimensional arrangement. In another embodiment, the method further comprises placing solid pieces as markers at specified intervals in the alignment. The markers

may have a different size from that of the solid pieces with probes. In an embodiment, each solid piece has one type of probe immobilized thereon, and a designated number of solid pieces for each type of probe are prepared. Additionally, the alignment of solid pieces may be conducted in an array selected from the group consisting of a capillary, a groove, and an optical cell.

In an embodiment of the method, the alignment of solid pieces may be conducted by (i) placing the probe-immobilized solid pieces on a sheet having a hole through which one solid piece can pass, said sheet being placed on a movable base having a through-hole leading to the interior of the array, said movable base being positioned where the hole of the sheet does not communicate with the through-hole of the movable base; (ii) trapping one of the solid pieces in the hole of the sheet; (iii) removing the remaining solid pieces from the sheet; (iii) moving the movable base to a position where the hole of the sheet communicates with the through-hole of the movable base; (iv) transferring the trapped solid piece to the array via the through-hole; and (v) repeating steps (i) through (iv) until the probe-immobilized solid pieces are aligned in the designated order in the array.

In another embodiment of the method, the alignment may be conducted by (i) placing the probe-immobilized solid pieces on a sheet having a hole through which one solid piece can pass, said hole leading to the interior of the array, said hole being closed with a valve; (ii) trapping one of the solid pieces in the hole of the sheet; (iii) opening the valve to transfer the trapped solid piece to the array, and (iv) repeating steps (i) through (iii) until the probe-immobilized solid pieces are aligned in the designated order in the capillary, groove, or optical cell.

In yet another embodiment of the method, the alignment may be conducted by (i) placing the probe-immobilized solid pieces in wells, each well containing a single type of probe-immobilized solid pieces, each well having a hole through which one solid piece can pass, said hole being closed; (ii) trapping one of the solid pieces in each hole of each well; (iii) opening and closing each hole after moving the wells in a designated order to transfer each trapped solid piece to an array; (iv) moving the wells to align the probe-immobilized solid pieces in a next array; and (v) repeating steps (i)

through (iv) until a designated number of arrays are filled with the probe-immobilized solid pieces aligned therein.

In still another embodiment of the method, the alignment may be conducted by (i) placing the probe-immobilized solid pieces in a narrow tube; (ii) moving the solid pieces one by one with a solution flowing along the narrow tube, to transfer the solid piece to the array, and (iii) repeating steps (i) and (ii) until the probe-immobilized solid pieces are aligned in the designated order in the array.

Additionally, in an embodiment, the alignment may be conducted by (i) placing the probe-immobilized solid pieces in sections, each section containing a single type of probe-immobilized solid pieces, each section having a hole through which one solid piece can pass, said hole being closed; (ii) trapping one of the solid pieces in each hole of each section; (iii) opening and closing each hole after moving the sections in a designated order to transfer each trapped solid piece to a groove; (iv) repeating steps (i) through (iii) until the probe-immobilized solid pieces are aligned in the groove in order; and (v) transferring the aligned probe-immobilized solid pieces to an array wherein the solid pieces are placed close together.

In the above, each embodiment can exhibit at least one of the aforesaid advantageous effects.

The present invention can be applied to other aspects, including a probe array for analyzing a sample solution passing therethrough, and various apparatuses for manufacturing a probe array.

The present invention will be explained by the following examples. A probe array of the present invention can be commonly explained either with DNAs, proteins, peptides or other biological materials. Accordingly, DNAs are used for explanation in the following examples.

In a DNA probe array according to the present invention, solid probes are held either one-dimensionally in a capillary or two-dimensionally in a small area of an optical cell. The capillary is mainly used in the Examples for convenience of explanation. Round beads are used as the fine particles in the Examples but any particles having cubic or other shapes can be used. Beads having a diameter of 1-300 microns can be used; however, beads having a diameter of 20 microns are mainly used

in the Examples. Further, glass or plastic beads are normally used; however, metal materials such as gold can also be used. Plastic beads are used here.

[Example 1]

Figure 1 shows an example of a probe array according to the present invention, wherein numeral 101 is an inlet for solution and sample, 102 is an outlet, 103 is a capillary for holding probe array, 104 are marker beads, 105 is a bead with probe, and 106 are dummy beads. The diameter of the beads with immobilized probes is 20 microns and the inner diameter of the capillary 103 is 25 microns. In this Example, about 20 dummy beads 106 are aligned on both ends and 999 beads 105 are aligned between them. Every 10^{th} bead is a black bead 104 and every 100^{th} bead is a red bead for a total of 99 marker beads and 900 probe beads, that is, 900 different kinds of probes can simultaneously be used for tests. These beads could be aligned in a 2 mm length if densely packed; however, in this example, for hybridization and other considerations, the beads were more loosely packed and held in a 5 mm length. The retention length can be longer or shorter than the above described range (e.g., in the range of 2-10 mm per 1000 beads). However, an excessively long length increases the amount of sample needed, while an excessively short length causes a problem in handling. Moreover, a sample may be not adequately hybridized. The volume of the reaction area is about 2.3 n liters. Stoppers are placed in both ends to prevent the beads from flowing-out. The sample and washing fluid are introduced and discharged through these ends via the inlet 101 and the outlet 102. The probe array is advantageously compact and easy to handle since as many as 10,000 probes can be held in an area of 20-30 mm in length.

The irradiating laser beam 206 and the probe holding capillary 202 are relatively scanned and the resulting fluorescence is measured using a fluorescence detection device, for example, as shown in Figure 2. In Figure 2, numeral 201 is a bead with probe, 202 is a capillary for holding probe array, 203 is a plate to move probe array, 204 is a point of irradiation and emission, 205 is a lens, 206 is a irradiation laser beam, 207 is a optical filter, 208 is a lens, 209 is a laser source, 210 is a detector, 211 is a controller for data processing and detector, and 212 is a indicator. Different probes are readily identifiable by the aid of marker beads placed every 10 beads 201. Marker beads can be colored differently to identify different probes, or alternatively, each group of 10 beads

with probes can be colored differently. Of course, in this case, colors would have to be chosen so as not to have a wavelength which would interfere with the fluorescence detection.

[Example 2]

This Example relates to a method and an apparatus in which beads are aligned in a capillary one at a time in predetermined order. Figures 3a-3g show an example of a device to make the bead array. In these figures, numeral 301 is an outlet for solution and beads, 302 is an inlet for solution, 303 is a cover plate, 304 is a bead with probe, 305 is a hole for bead trapping, 306 is a capillary for bead alignment, 307 is a capillary holding base, 308 is a trapped bead, 309 is a nozzle for bead supply, and 310 is a stopper. For convenience of explanation, beads are aligned in one capillary in this Example; however, for practical use, a multiple number of holes on a sheet and a multiple number of capillaries are used. Step 1 (Figure 3a): Beads with the first probe (probe bead #1, 304) are introduced with a solvent into the cell 303 having the sheet 311 with a hole at the bottom. The beads are precipitated and the solvent is moved back and forth and right and left to drop one of the beads 305 into the hole. Step 2 (Figure 3b): The remaining beads are removed with the solvent 302 via the outlet 301 and washed. Only the bead which dropped into the hole remains in the cell. In this case, the solvent may be blown out of the port at a right angle to the sheet to remove these beads near the port and leave the one bead in the sheet hole to be introduced into the capillary in step 3. The bottom of the hole is closed off by the block 307. The capillary for the alignment of the beads is fixed to this block, but in steps 1 and 2, the axis 306 of the capillary and the hole are not aligned such that the bead 305 is retained in the hole. Step 3 (Figure 3c): The block 307 and the sheet 311 are moved relative to each other to align the axis of the capillary and the hole. Probe bead #1 (305) is introduced into the capillary by suction from the other end or with pressure applied from the solution injection side. In this case, the relative movement of the block and the sheet is about the same order as the diameter of the hole, for which a piezoelectric element is successfully used. Step 4 (Figure 3d): The block 307 and the sheet 311 are relatively moved so that the axis 306 of the capillary and the hole 308 are again out of alignment. Step 5 (Figure 3e): Beads with the second probe (probe bead #2, 320) are introduced into the cell 303 and one of

them 321 is dropped into the hole. Step 6 (Figure 3f): Excess beads other than the bead in the hole are removed from the cell in the same manner as in step 2. Step 7 (Figure 3g): The block and the sheet are moved relatively to align the axis of the capillary and the hole such that the probe bead #2 (321) can be introduced into the capillary. As a result, the bead with probe 1 (probe bead 1) and the bead with probe 2 (probe bead 2) are aligned in the capillary. By repeating these steps, a bead array with probes having a desired order can be produced.

The capillary used here can be taken out and used as a probe array holder during measurement, or a probe array holder can be made separately and attached to the bottom part of the capillary to which the bead array is transferred. In this Example, the probe array holder shown in Figures 4a and 4b is used. In these figures, numeral 401 is a base with a bead array holding groove, 402 is a solution outlet capillary, 403 is an inlet for beads and various solutions, 404 is a groove for bead alignment, 405 is a bead with probe, 406 is a stopper, and 407 is an upper window. A sample solution is injected from the left side (403) of the Figure. After sufficient hybridization, a washing liquid is injected from the right side (402) to remove the unreacted sample portion. After mounting the probe array holder onto a measuring unit, each bead is irradiated with a laser beam and emitted fluorescence is detected. Of course, instead of emitted fluorescence from laser beam irradiation, emitted light produced by a chemical emitting reagent can also be detected. Any detection method which can detect the presence and absence of hybridization can be used.

In this Example, the invention is explained with only one capillary fixed to the block; however, it is possible to produce a large number of probe arrays simultaneously using a multiple number of capillaries. In that case, it is naturally understood that the number of holes on the sheet has to be increased as the number of capillaries increases.

[Example 3]

This example is for an apparatus in which a bead delivery device 504 having holes (or wells) to keep various kinds of beads separately to transfer them to a bead arraying plate 512 having grooves 507 on it or a capillary for aligning the beads according to the predetermined order as a probe bead array. At first solutions containing different kinds of probe beads placed in wells of a titer plate are transferred

one after another in a predetermined order into designated wells (holes) of a bead delivery device such that the beads are aligned in a groove produced in a plate or a capillary (Figures 5a, 5b, and 5c). In these figures, numeral 501 is a pipetter/injector, 502 is a titer plate which has wells 503 containing probe beads, 504 is a bead delivery device with holes, 505 is a hole which holds probe beads being delivered to a groove, 506 are arrayed probe beads, 507 is a groove in which various kinds of probe beads are aligned, 508 is a probe bead, 509 is a probe bead trapped in a hole, 510 is a piezoelectric element, 511 is a movable valve, and 512 is a holding base. The beads are suctioned from the wells in the titer plate 502 with the pipetter 501 and moved into a transfer well 505. The hole 520 for trapping a bead is open at the bottom of the well. One of the beads 509 (a multiple number of the beads if a multiple number of the holes are provided) injected into the well 505 drops into the hole 520, and the presence of the dropped bead is optically confirmed. Then, excess beads are recovered or removed from the well by flushing beads out with washing liquid. The valve 511 which can be driven by a piezoelectric element 510 or the like is placed between the bead trapping hole 520 and the groove 507 or the capillary. A bead can be transferred to the groove or capillary side by moving the valve. Actual bead movement is controlled by a liquid flow. Of course, the bead can also be transferred by moving the plate 504 to align the hole and the groove or the center of the capillary. Once the bead is fully transferred, the valve is moved back or the relative position of the hole and the capillary is shifted so that the bead is trapped in the hole. Beads with the next probe are introduced into the trapping site using a pipetter. The steps above are repeated to produce a bead array. The resulting bead array 506 is used as it is, or transferred to another container while maintaining the alignment and used as a probe array.

The steps above can be carried out in a system having a multiple number of holes to save time in array production, or to simultaneously produce a multiple number of the same arrays.

[Example 4]

In Example 2, one kind of probe bead at a time is aligned using a bead delivery device with one hole. In this example, a multiple number of wells in a bead delivery device are used to segmentally hold multiple kinds of probes bead in order to improve

productivity. As shown in Figure 6, a multiple number of rectangular wells 603 are placed on a rotary disk 601. In Figure 6, numeral 601 is a disk-type bead holding plate for delivering beads, 602 is a rotary axis, 603 is a groove for bead holding, and 604 is a hole for bead holding. The bottom of each well is fitted with a sheet with holes as described in Example 1 at the bottom. The lower part of the rotary disk having the sheets has contact with a block, which holds capillaries, to prevent dropping of the beads trapped in the holes. When the rotary disk is moved and the holes and the axis of the capillaries are aligned, the probe beads are transferred into the capillaries in the same manner as described in Example above. The number of the holes corresponds to the number of the capillaries. The holes and the capillaries are correspondingly positioned; however, in order to prevent shearing upon rotation, a controlling mechanism is provided, in which the block with capillaries is moved in the axial 602 direction of the disk using a tracking technique similar to that used for CD-ROMs. In this example, a rotating board having a diameter of 16 cm is used. Wells 603 (1 mm wide and 30 mm long) are located at a position 5 cm from the axis of the disk. The pitch of the wells is 2 mm and about 150 wells can be radially placed on the disk. The sheet with holes is spread under the wells and the pitch of the holes is 2 mm. In this example, a total of 10 holes are aligned so that probe bead arrays can be made in 10 capillaries. Of course, the number of capillaries and the number of probe arrays producible at one time can be changed as required.

The rotary plate rotates in two rotation modes; a high speed rotation mode and a low speed but highly accurate mode. Beads are introduced into the well with a solution. The beads are dropped into the holes by moving the disk and flowing the solution out of the holes. Next, excess beads are moved to bead holders located on the end of the wells by centrifugal force and by water flow by rotating the disk in the high speed rotating mode. The disk is stopped, then, disk rotation is set to the highly accurate mode so that the capillaries and the probe beads #1 align. A shutter at the bottom of the disk is opened and the block which is holding the capillaries is brought into contact with the rotary plate such that the wells carrying probe bead #2 are moved to the position of the capillaries. The beads are sequentially transferred into capillaries to produce probe bead arrays in a designated order. A large number of probe beads can be aligned and held in

capillaries by exchanging the disk or the probe beads to be placed in the wells and repeating the above described steps. The position of a specific probe in a resulting probe bead array can be conveniently confirmed by changing the color of beads in the arrays every 10 beads.

[Example 5]

This example relates to a method and an apparatus for the alignment of probe beads into a capillary one by one in a designated order using a liquid flow. Figure 7 shows a conceptual view of this example. In this figure, numeral 701 is a bead solution reservoir, 702 is a bead with probe, 703 is a transfer tube, 704 is a sheath flow cell, 705 is a transfer liquid, 706 is a capillary tube for transfer, 707 is a capillary for bead array alignment; 708 is a supporting base, and 709 is a solution outlet tube. Beads 702 with probes are pumped into the transfer capillary tube 707. The end of the capillary tube is inserted into a liquid flow formed with the transfer liquid 705 in the sheath flow cell 704, and the beads are released into the liquid flow one by one, and virtually constant intervals. However, to stabilize the release, ultrasonic waves are applied to that portion of the capillary holding the beads to form knots along the axis of the capillary. The beads are released one by one into the liquid flow at designated intervals by controlling conditions such as the intensity of the ultrasonic waves.

[Example 6]

In the examples above, one bead corresponds to one kind of probe. However, in order to check uniformity of hybridization reactions or to improve detection sensitivity, it is appropriate to use a multiple number of beads for one kind of probe. It is not necessary that the same number of beads be used for all probes. If the number varies held in a capillary for making a probe array, however, colored beads or beads of a different size have to be inserted between bead groups with different probes as markers. This example is shown in Figure 8. In this figure, numeral 801 is a large size dummy beads, 802 is a probe bead, 803 is a large size marker bead, 804 is a capillary for probe holding, and 805 is a sample flow path. The apparatus for the production is virtually the same as described above, except that the size of the holes is several times larger than the size of the beads 802 so that a multiple number of beads 802 are trapped in the holes. Subsequent procedures are the same as described above.

Further, the bead array of this example can be easily produced if the liquid flow system described in Example 5 is used. A small number of beads are suctioned from a bead reservoir with a pipet and injected into the liquid flow. Although the number cannot be confirmed, the injected beads can be sequentially placed into the capillary 804. Prior to the injection of another kind of beads, a colored bead or a bead of a different size (801) is injected as a marker so that the position and the kind of probe of individual beads can be identified.

[Example 7]

The previous example is a method for the production of a probe array in which probe beads are aligned in a capillary. This example as shown in Figures 9a and 9b discloses a method and an apparatus in which beads are first aligned in a groove produced on a plane surface, then congregated into a probe array or transferred into a capillary to produce a probe array. In Figures 9a and 9b, numeral 901 is a plate having wells, 902 is a well for a bead reservoir, 903 is a bead holding hole, 904 is a sheet with holes and is usually attached to the plate 901, 905 is a base array production holder with grooves for aligning beads, 906 is a fine groove for probe bead alignment, 907 is a bead with probe, and 908 is a capillary for bead array. First, a bead array production holder 905 having a multiple number of grooves 906 on a plane surface is prepared. Beads 907 with probes are aligned in each groove and transferred into a capillary 908 or the like while maintaining their alignment, then the beads aligned in the multiple number of grooves are introduced into different capillaries and used as a probe array. A plate attached with a sheet having holes (901, 904) is placed on top of the bead array production holder wherein beads are trapped in the holes and transferred into the above described grooves. As shown in Figure 9, this plate with a sheet has wells (bead reservoirs 902) orthogonal to the grooves on the beads array production holder, and the holes 903 holding beads are through holes and opened for the fine groove. The apparatus does have a multiple number of grooves, but beads with different probes are injected into different wells in the plate and held in different holes. The plate attached with a sheet and the plate having grooves are used in close contact but can slide each other. At the start, the holes 903 of the sheet and the grooves 906 for the bead array production holder are not aligned. Beads with probes are supplied into different wells

902 of the plate above the sheet with holes for each kind of probe. One bead drops into one hole and retained there because the bottom of the hole is closed at this state. When the holes of the sheet and the grooves of the bead array production holder are aligned, the beads drop one by one from individual holes into the grooves 906. Since different probe beads drop into one groove from different positions, a variety of probe beads are retained in a groove. The beads are placed virtually at the same intervals as those in the bead reservoir 902 on the sheet with holes. In this example, the interval is 2 mm. A total of 50 beads are dropped into each groove of the bead array production holder in this example. Also, 10 bead arrays can be simultaneously produced in this example, but the number can be increased as desired. After dropping the beads, the positions of the sheet with holes 903 and the grooves 906 of the bead array production holder are shifted to seal up the grooves, after which the beads are introduced into the capillary 908 with a liquid flow. The number of different kinds of probes can be arrayed by repeating the above described steps.

In this example, a one-dimensionally aligned probe bead array is disclosed; however, naturally, probe arrays having many more kinds of probes can be produced by arranging a multiple number of these arrays or by two-dimensionally aligning these arrays.

[Example 8]

In this example, a probe bead array holder comprises cells which consist of a plate with one-dimensionally or two-dimensionally distributed holes and a cover glass. In Figure 10a, 10b, and 10c, numeral 1001 is a microtiter plate-type bead array holder, 1002 is a spacer, 1003 is hole which makes a bead array cell with a cover glass, 1004 is a bead with probe, 1005 is a cover glass, 1006 is a solution outlet, 1007 is a solution inlet, 1008 is a laser beam, 1009 is a lens, and 1010 is a detector. This resembles a micro titer plate. A small number of beads are suctioned from a titer plate in which probe beads are held, and dispensed into holes (cells) 1003 of the plate 1001. The beads 1004 are dispensed into the holes at designated positions according to the kind of probe to produce a microtiter plate-type bead array with probe beads. After the beads are dispensed, the cover glass 1005 which is optically transparent and does not interfere with the measurement of fluorescence or chemical emission is placed on top to produce

a cell array. The space between the cover glass and walls, which segment the cells of the microtiter plate-type cell array, is smaller than the size of the beads so that the beads cannot move out. The reaction solution or the like can flow through the cells freely. For use, the cells are turned upside down to make the glass side down. In this case, the beads on the glass surface are sufficiently in contact with the reaction solution independent of the depth of the cells and the probes undergo hybridization with the target.

[Effectiveness of the Invention]

As described above, according to the present invention, a large number of probe arrays for peptides or DNAs can be produced by a simple procedure. The process to immobilize probes on the surface of a solid and the process to align probes are separated, so that both processes can be optimized. As a result, immobilized probes which are homogeneous and not easily removable from the surface of the solid can be produced, then an array having the required kinds of probes can be readily produced by aligning the beads in a designated order. Also, a fine probe array, which is difficult to make by a conventional method, can be produced by reducing the size of the beads. A probe array with new components can be produced simply by preparing the required DNA probes, immobilizing them on the surface of beads and setting the probe beads onto a production apparatus, and thus arrays as requested by users can be provided any time. By aligning a multiple number of beads carrying the same probes, statistical averages can be obtained to analyze reproducibility and quantitativeness, and reliable measurements can be carried out. Furthermore, the reaction is quick and highly sensitive because the surface area for the reaction is larger than that in conventional DNA chips or the like being retained on a plane. The size of the beads can vary between 1 micron to 300 microns so that high density probe arrays can be readily produced if necessary. For example, by using 6-micron beads, 1,500 probes can be aligned in a 10-mm length in a capillary, or more than 1,000,000 probes can be retained in an area of 1 cm^2 if a two-dimensional probe array holder is used.

A multiple number of arrays having the same probe alignment can be produced by an extremely simple procedure and thus the arrays are also suitable for mass production.

It will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present invention. Therefore, it should be clearly understood that the forms of the present invention are illustrative only and are not intended to limit the scope of the present invention.

WHAT IS CLAIMED IS:

1. A method for producing a probe array, comprising the steps of:

selecting plural types of probes of interest;

immobilizing the plural types of probes on surfaces of different solid pieces; and

aligning the probe-immobilized solid pieces in a designated order to obtain a probe array for analyzing a sample solution passing therethrough.

2. The method according to Claim 1, wherein the probes are polynucleotides, peptides, or proteins.

3. The method according to Claim 1, wherein the solid pieces are beads.

4. The method according to Claim 3, wherein the beads are fine particles.

5. The method according to Claim 1, wherein the alignment of the solid pieces is a one-dimensional arrangement or a two-dimensional arrangement.

6. The method according to Claim 1, further comprising placing solid pieces as markers at specified intervals in the alignment.

7. The method according to Claim 1, wherein each solid piece has one type of probe immobilized thereon, and a designated number of solid pieces for each type of probe are prepared.

8. The method according to Claim 6, wherein the markers have a different size from that of the solid pieces with probes.

9. The method according to Claim 1, wherein the alignment is conducted in an array selected from the group consisting of a capillary, a groove, and an optical cell.

10. The method according to Claim 9, wherein the alignment is conducted by (i) placing the probe-immobilized solid pieces in a well on a sheet having a hole through which one solid piece can pass, said sheet being placed on a movable base having a through-hole leading into the array holder, said movable base being positioned where the hole of the sheet does not communicate with the through-hole of the movable base; (ii) trapping one of the solid pieces in the hole of the sheet; (iii) removing the remaining solid pieces from the sheet; (iii) moving the movable base to a position where the hole of the sheet communicates with the through-hole of the movable base; (iv) transferring the trapped solid piece to the array via the through-hole; and (v) repeating

steps (i) through (iv) until the probe-immobilized solid pieces are aligned in the designated order in the array.

11. The method according to Claim 9, wherein the alignment is conducted by (i) placing the probe-immobilized solid pieces in a well on a sheet having a hole through which one solid piece can pass, said hole leading to the interior of the array, said hole being closed with a valve; (ii) trapping one of the solid pieces in the hole of the sheet or in the well; (iii) opening the valve to transfer the trapped solid piece to the array, and (iv) repeating steps (i) through (iii) until the probe-immobilized solid pieces are aligned in the designated order in the capillary, groove, or optical cell.

12. The method according to Claim 7, wherein the alignment is conducted by (i) placing the probe-immobilized solid pieces in wells, each well containing a single type of probe-immobilized solid pieces, each well having a hole through which one solid piece can pass, said hole being closed; (ii) trapping one of the solid pieces in each hole of each well; (iii) opening and closing each hole after moving the wells in a designated order to transfer each trapped solid piece to an array; (iv) moving the wells to align the probe-immobilized solid pieces in a next array; and (v) repeating steps (i) through (iv) until a designated number of arrays are filled with the probe-immobilized solid pieces aligned therein.

13. The method according to Claim 9, wherein the alignment is conducted by (i) placing the probe-immobilized solid pieces in a narrow tube; (ii) flowing the solid pieces one by one with a solution along the narrow tube, to transfer the discharged solid piece to the array, and (iii) repeating steps (i) and (ii) until the probe-immobilized solid pieces are aligned in the designated order in the array.

14. The method according to Claim 7, wherein the alignment is conducted by (i) placing the probe-immobilized solid pieces in sections, each section containing a single type of probe-immobilized solid pieces, each section having a hole through which one solid piece can pass, said hole being closed; (ii) trapping one of the solid pieces in each hole of each section; (iii) opening and closing each hole after moving the sections in a designated order to transfer each trapped solid piece to a groove; (iv) repeating steps (i) through (iii) until the probe-immobilized solid pieces are aligned in the groove

in order; and (v) transferring the aligned probe-immobilized solid pieces to an array wherein the solid pieces are placed close together.

15. A probe array for analyzing a sample solution passing therethrough, comprising:

plural types of probes immobilized on surfaces of different solid pieces; and

an array wherein the probe-immobilized solid pieces are aligned in a designated order.

16. The probe array according to Claim 15, wherein the probes are polynucleotides, peptides, or proteins.

17. The probe array according to Claim 15, wherein the solid pieces are beads.

18. The probe array according to Claim 17, wherein the beads are fine particles.

19. The probe array according to Claim 15, wherein the alignment of the solid pieces is a one-dimensional arrangement or a two-dimensional arrangement.

20. The probe array according to Claim 15, further comprising solid pieces as markers at specified intervals in the alignment.

21. The probe array according to Claim 15, wherein each solid piece has one type of probe immobilized thereon, and a designated number of solid pieces for each type of probe are prepared.

22. The probe array according to Claim 20, wherein the markers have a different size from that of the solid pieces with probes.

23. The probe array according to Claim 15, wherein the array is selected from the group consisting of a capillary, a groove, and an optical cell.

24. An apparatus for manufacturing a probe array, comprising:

a sheet or well for supporting probe-immobilized solid pieces, said sheet or well having a hole through which one solid piece can pass;

a movable base on which the sheet or well is placed, said movable base having a through-hole leading to an array, said movable base being movable between a position where the hole does not communicate with the through-hole,

wherein a probe-immobilized solid piece is trapped in the hole, and a position where the hole communicates with the through-hole, wherein the probe-immobilized solid piece is discharged; and

an array detachably communicating with the through-hole, wherein the probe-immobilized solid pieces can be aligned in a designated order in the array.

25. An apparatus for manufacturing a probe array, comprising:

a sheet or cell for supporting probe-immobilized solid pieces, said sheet or cell having a hole through which one solid piece can pass, said hole leading to an array;

a valve for closing the hole, wherein when the hole is closed, a probe-immobilized solid piece is trapped in the hole, and when the hole is opened, the probe-immobilized solid piece is discharged; and

an array detachably communicating with the hole, wherein the probe-immobilized solid pieces can be aligned in a designated order in the array.

26. An apparatus for manufacturing a probe array, comprising:

a plate having a plurality of wells for supporting probe-immobilized solid pieces therein, each well corresponding to one type of probe-immobilized solid pieces, each well having a hole through which one solid piece can pass;

valves for closing the holes, wherein when each hole is closed, a probe-immobilized solid piece is trapped in the hole, and when each hole is opened, the probe-immobilized solid piece is discharged, said valves being manipulated in a designated order; and

a plurality of arrays communicating with the holes, wherein the probe-immobilized solid pieces can be aligned in a designated order in each array.

27. An apparatus for manufacturing a probe array, comprising:

a narrow tube for supporting probe-immobilized solid pieces therein;

an outer tube surrounding the narrow tube wherein a solution flows in a direction of discharging the solid pieces one by one; and

an array receiving the solid pieces discharged from the narrow tube, wherein the probe-immobilized solid pieces can be aligned in a designated order in the array.

28. An apparatus for manufacturing a probe array, comprising:

a plate having sections for supporting probe-immobilized solid pieces therein, each section containing a single type of probe-immobilized solid pieces, each section having a hole through which one solid piece can pass, said hole having a vale;

a second plate having grooves for receiving probe-immobilized solid pieces passing through the holes in a designated order; and

an array receiving the solid pieces transferred from the grooves, wherein the solid pieces are placed close together in order.

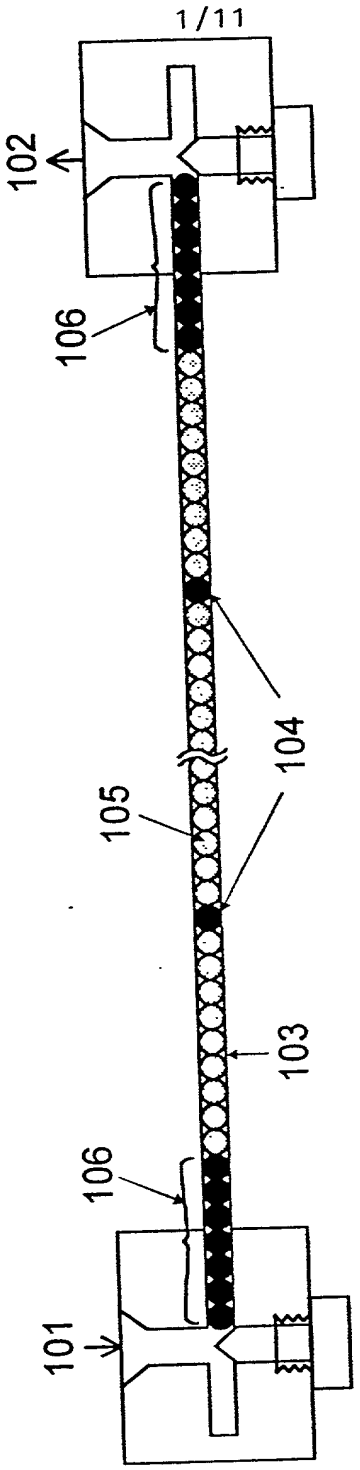


FIG. 1

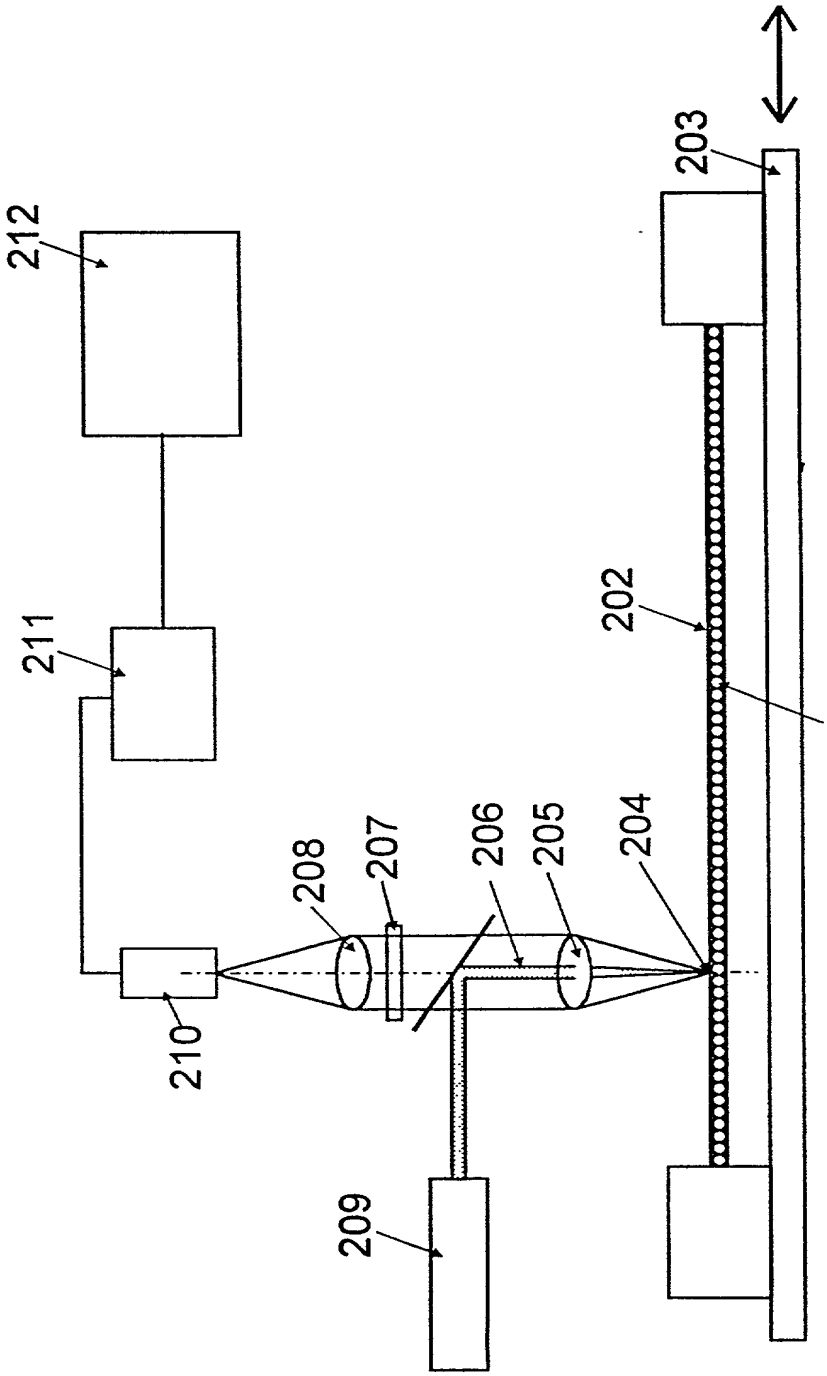


FIG. 2

FIG. 3.a

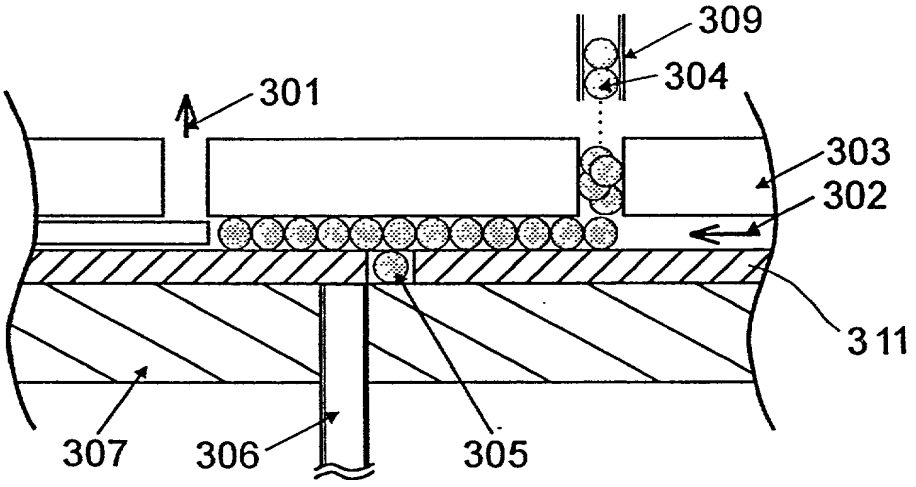


FIG. 3.b

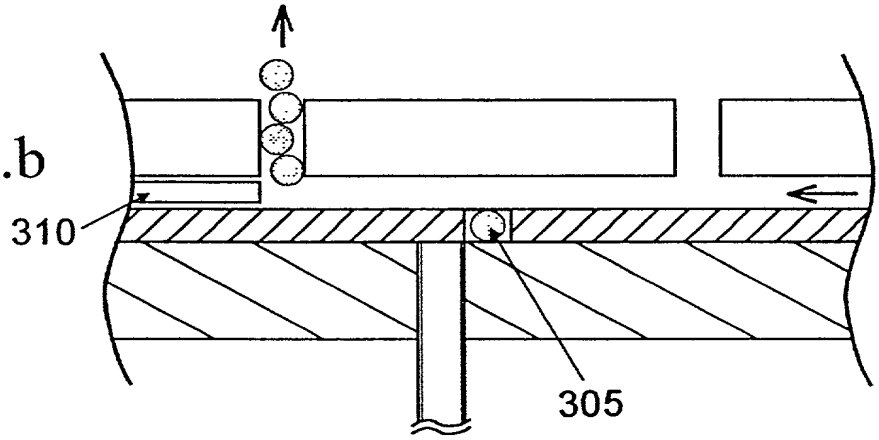


FIG. 3.c

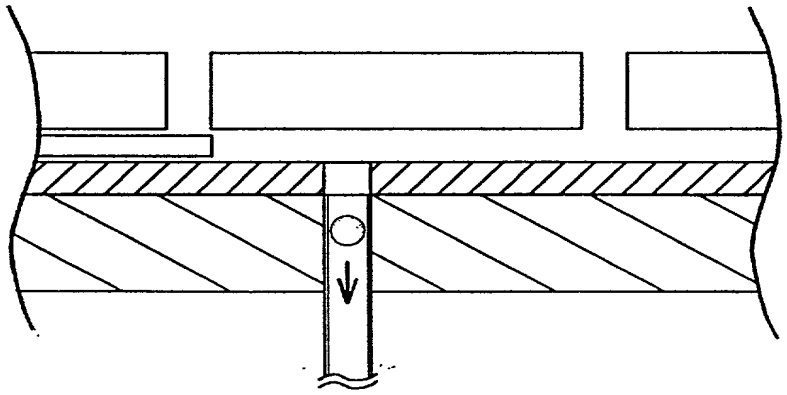


FIG. 3.d

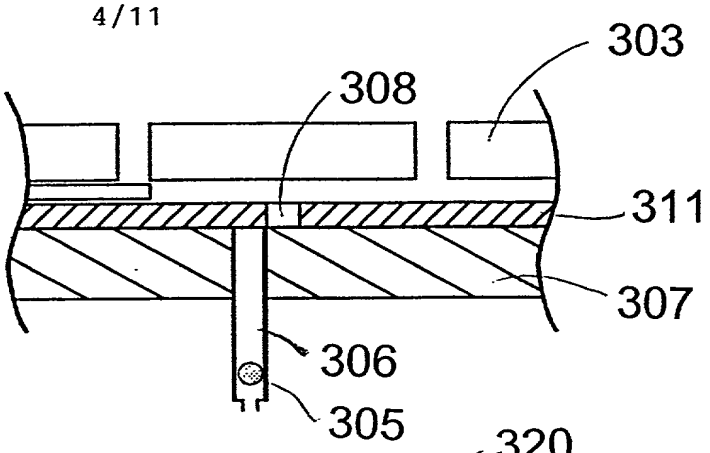


FIG. 3.e

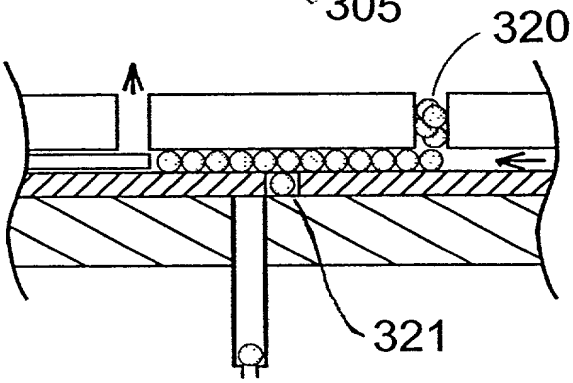


FIG. 3.f

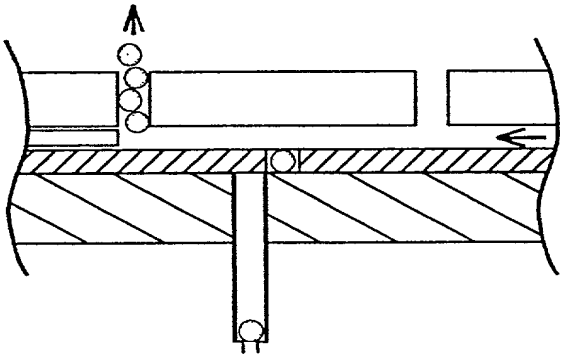


FIG. 3.g

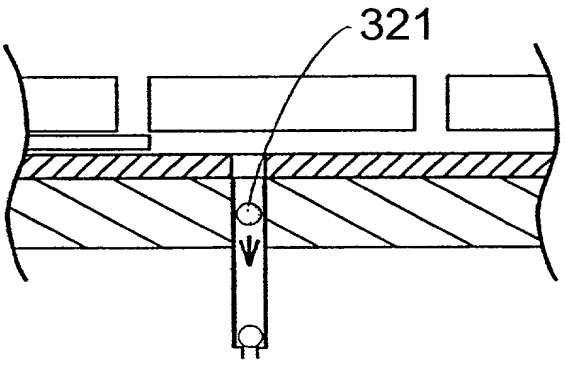


FIG. 4a

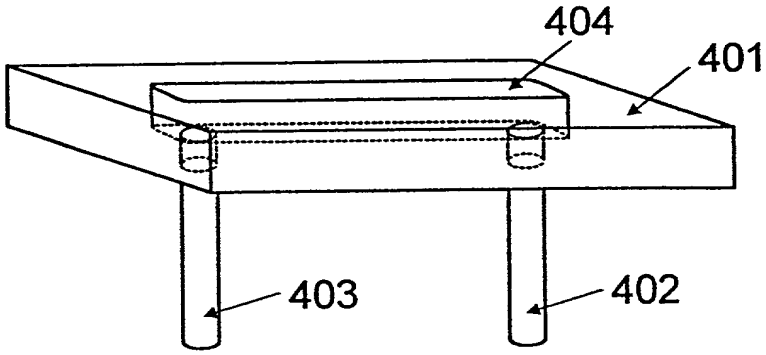
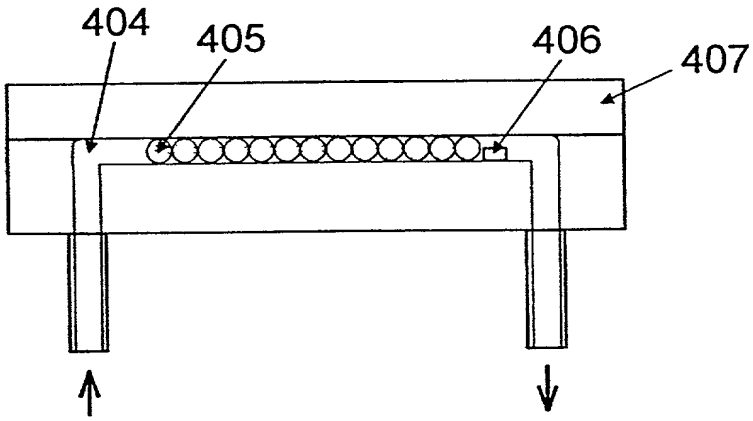


FIG. 4b



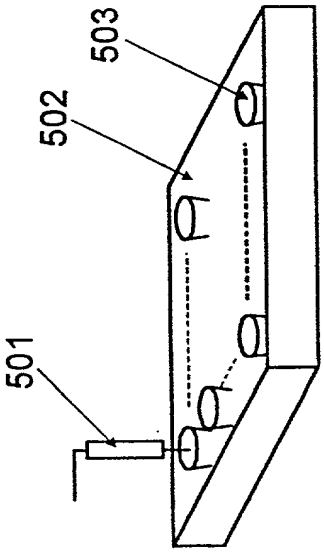


FIG. 5a

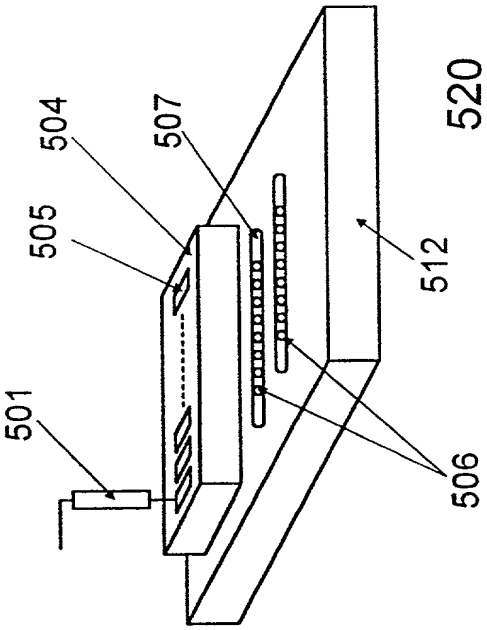


FIG. 5b

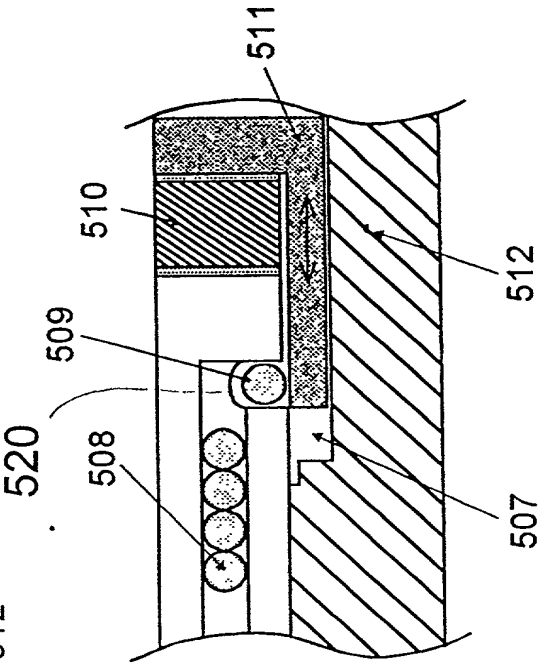


FIG. 5c

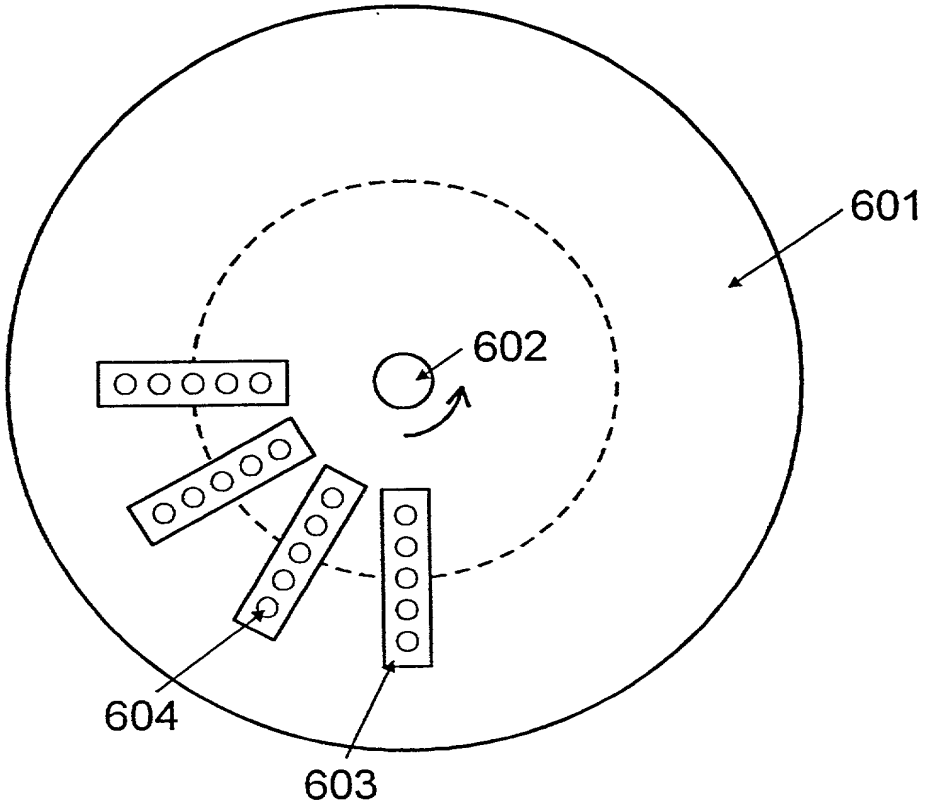


FIG. 6

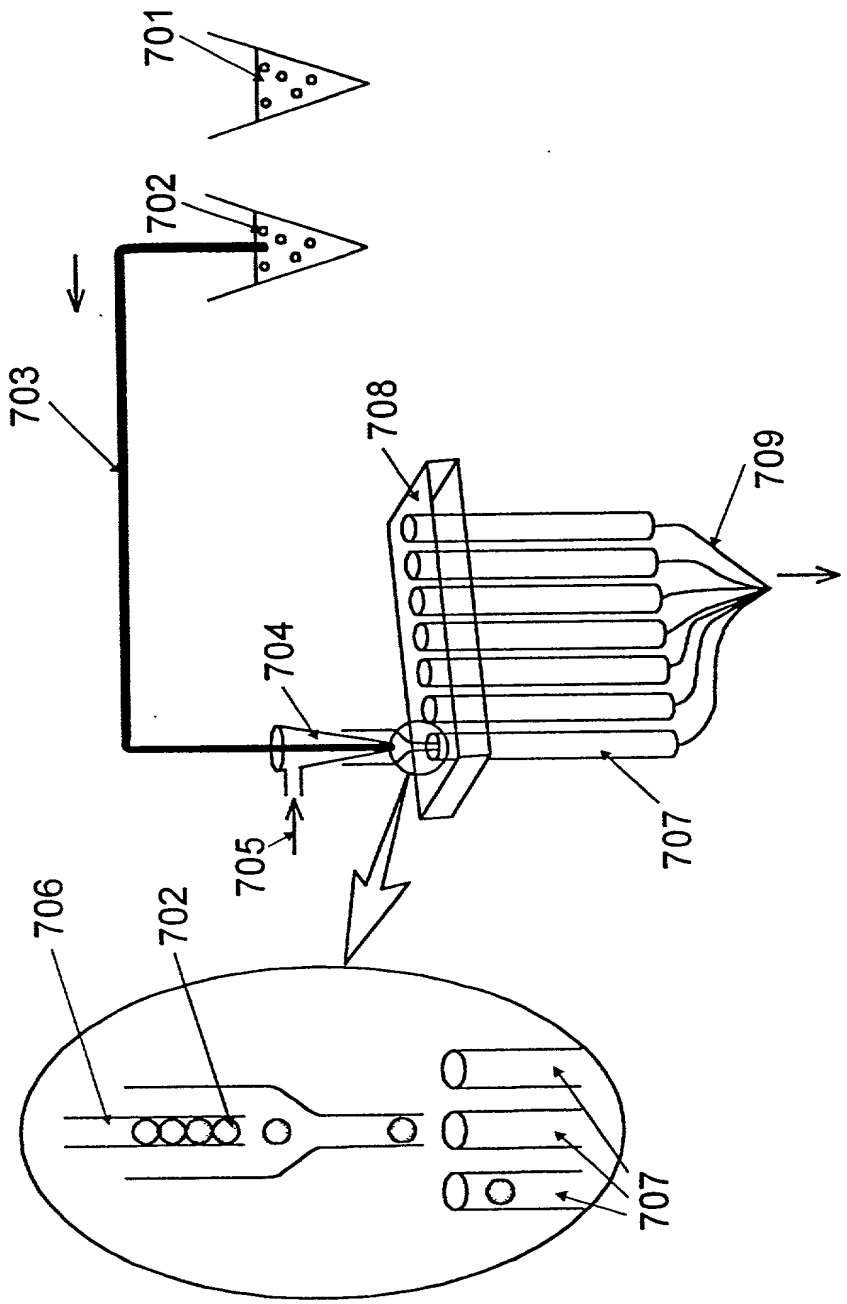


FIG. 7

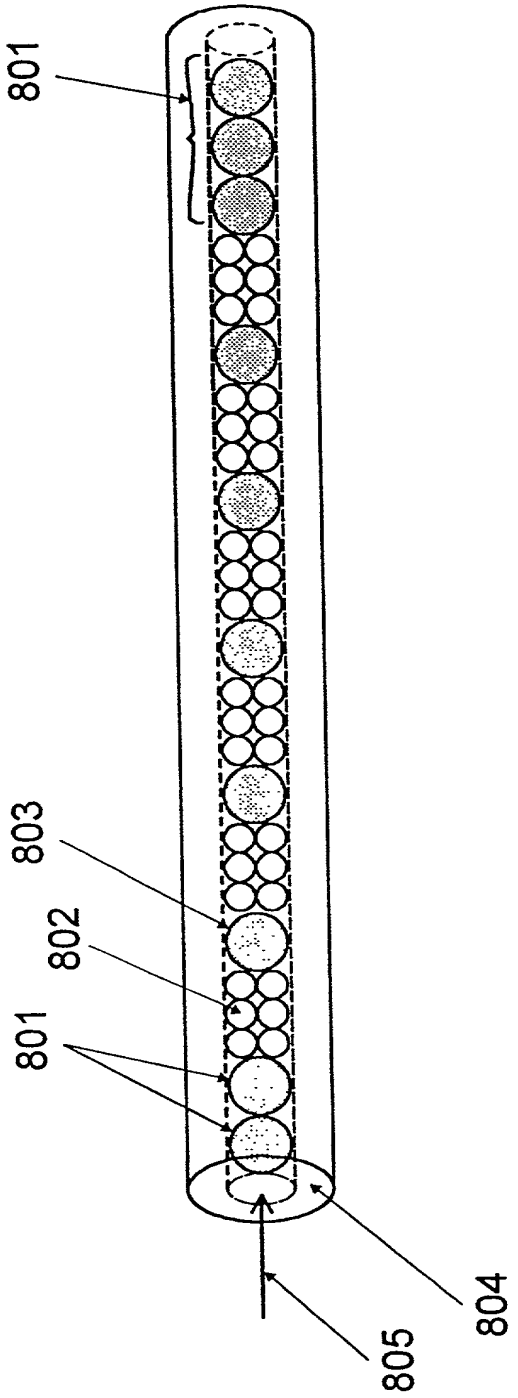


FIG. 8

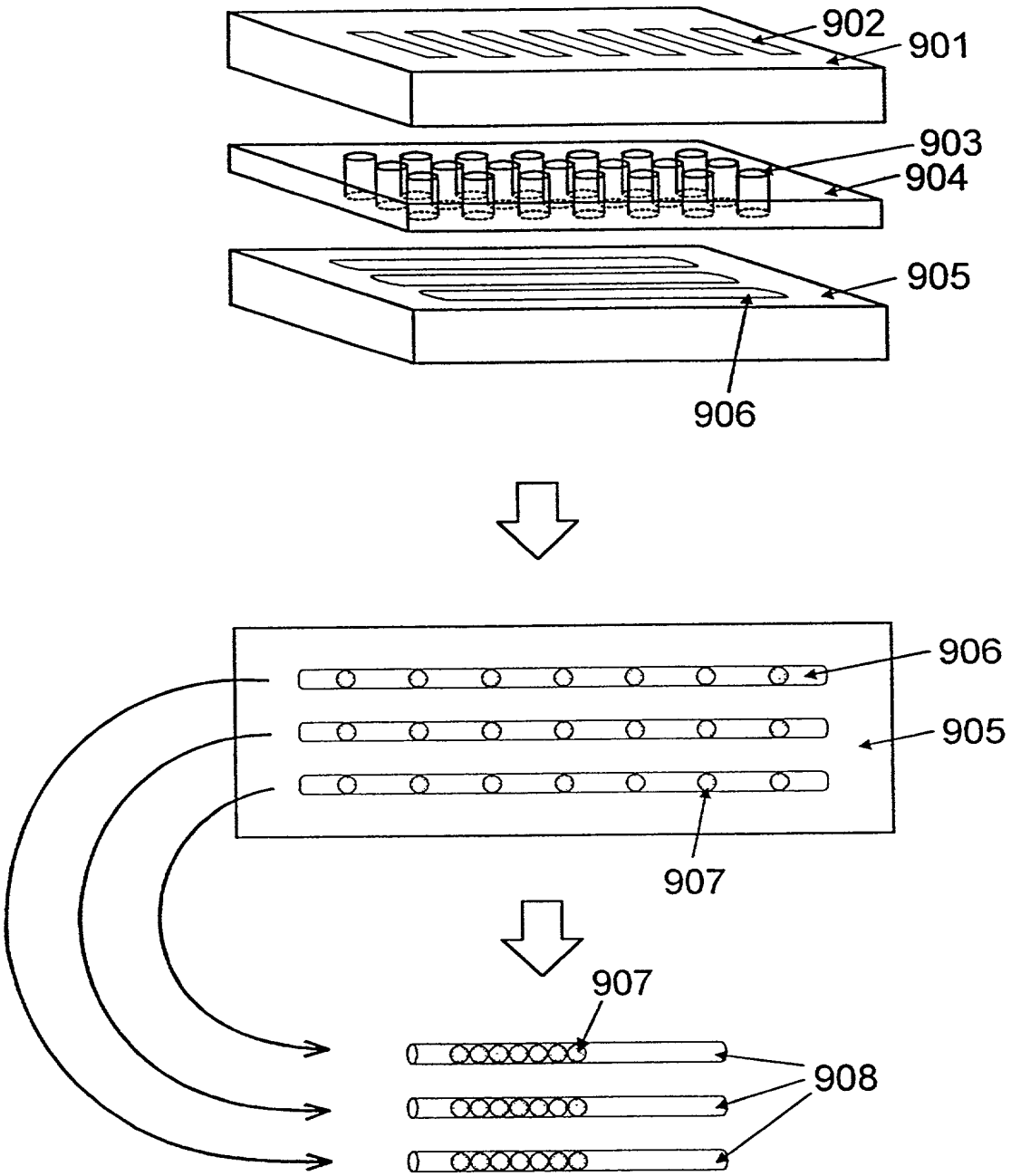


FIG. 9

FIG. 10a

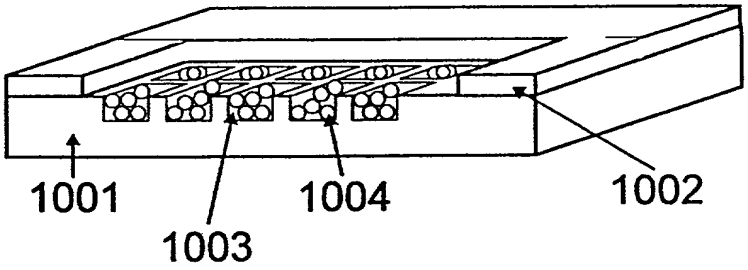


FIG. 10b

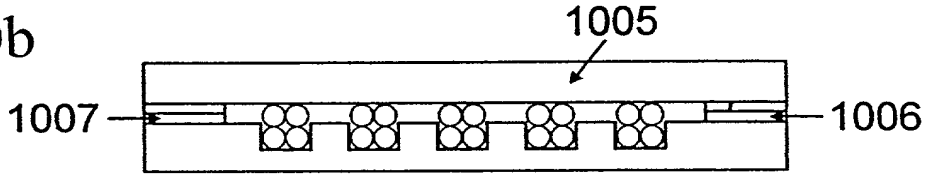
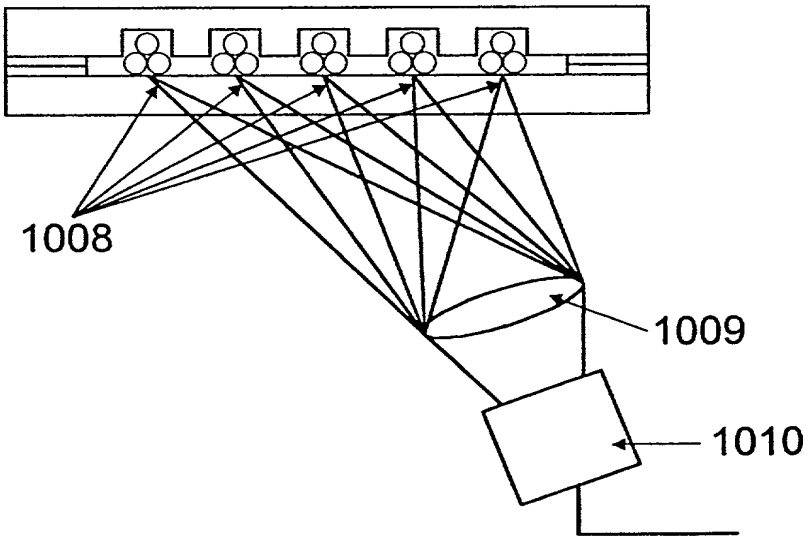


FIG. 10c



DECLARATION AND POWER OF ATTORNEY- USA PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD OF PRODUCING PROBE ARRAYS FOR BIOLOGICAL MATERIALS USING FINE PARTICLES; PCT Application No. PCT/US00/09685, filed in the U.S.A. Office on April 11, 2000; the documentation for entry into the U.S. national phase of which was filed on **September 18, 2001** as Application Serial No. **09/937,105**.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56;

I hereby claim the benefit under Title 35, United States Codes § 119(e) of any United States provisional application(s) listed below.

Application No.: **60/128,861** Filing Date: **April 12, 1999**

POWER OF ATTORNEY: I hereby appoint the registrants of Knobbe, Martens, Olson & Bear, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, California 92660, Telephone (949) 760-0404, **Customer No. 20,995**.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

100

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Inventor's signature Hideki Kambara

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Date Oct 25, 2001

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